Annals of Oncology

Annals of Oncology 26: 598–607, 2015
doi:10.1093/annonc/mdu566
Published online 8 December 2014

Efficacy and safety of biweekly i.v. administrations of the Aurora kinase inhibitor danusertib hydrochloride in independent cohorts of patients with advanced or metastatic breast, ovarian, colorectal, pancreatic, small-cell and non-small-cell lung cancer: a multi-tumour, multi-institutional phase II study

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; revised 24 October 2014; accepted 28 November 2014

Background: This multi-centre phase II trial assessed the activity, safety (CTCAE 3.0) and pharmacokinetics (PK) of the pan-Aurora kinase inhibitor danusertib hydrochloride (PHA-739358) in breast (BC), ovarian (OC), pancreatic (PC), colorectal (CRC), small-cell (SCLC) and non-small-cell lung (NSCLC) cancers.

Methods: Consenting adult patients with good performance and organ function with advanced/metastatic tumours who had failed systemic therapy were treated in independent, disease-specific cohorts with danusertib 500 mg/m^2 given as 24-h i.v. infusion every 14 days with until progression or unacceptable toxicity. A two-stage design was applied. Primary end point was the progression-free rate (PFR) at 4 months (RECIST1.1).

Results: A total of 223 patients were enrolled with 219 actively treated. The median relative dose intensity of danusertib was similar for all tumour types (84.6%–99.6%). The median number of biweekly treatment cycles ranged from 3 to 4/patient (maximum 5–40 cycles/entity) and the median treatment duration varied between 7.6 and 10.0 weeks per histotype. Danusertib did not meet pre-specified protocol criteria for clinically relevant activity in any of the treated cancers. The PFR at 4 months was 18.4% in BC, 12.1% in OC, 10.0% in PC, 10.4% in NSCLC (all histotypes), 16.1% in squamous NSCLC and 0% in SCLC and CRC. Some radiological and/or biochemical indication of antitumor activity was seen in BC, OC, PC and NSCLC, including two confirmed partial responses. The most frequent drug-related non-laboratory adverse events (AEs) were fatigue/asthenia, nausea, diarrhoea, anorexia, vomiting, alopecia, constipation and pyrexia. Common laboratory AEs included haematological toxicity, hypalbuminaemia and increases in liver enzymes. Treatment was discontinued due to AEs in only 5.5% of patients. Plasma concentrations of danusertib were in line with results from earlier studies.

Conclusion: Single-agent danusertib did show only marginal anti-tumour activity in common solid tumours after failure of prior systemic therapies. The safety and PK profile was consistent with previous experience.

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†Previous Publications: Gallerani E, Delord JP, Schöffski P, et al. Phase II study of Danusertib (D) in advanced/metastatic breast and ovarian cancers (BC, OC). Proceedings of the Annual Meeting of the American Society of Clinical Oncology 2010 (abstract 5014); Laffranchi B, De Jonge MJ, Bajetta E, et al. Phase II study of Danusertib (D) in advanced/metastatic colorectal and pancreatic cancers (CRC, PC). Proceedings of the Annual Meeting of the American Society of Clinical Oncology 2010 (abstract e13558); Gauler E, Delord JP, Schöffski P, et al. Phase II study of danusertib (D) in advanced/metastatic non-small cell lung cancers (NSCLC). Proceedings of the Annual Meeting of the American Society of Clinical Oncology 2013 (abstract e19138).

Clinical trial number: 2006-003772-35.
Key words: aurora kinase inhibitor, breast cancer, ovarian cancer, colorectal cancer, pancreatic cancer, lung cancer

introduction

The Aurora kinases belong to a family of highly conserved serine/threonine protein kinases. They play an essential role as key mitotic regulators, controlling entry into mitosis, centrosome function, chromosome assembly and segregation [1]. Three Aurora isoforms are known in man [2]. Aurora kinase A is essential for the timely entry into the M phase of the cell cycle, maintaining spindle bipolarity and chromosome segregation; Aurora kinase B is required for chromosome condensation, alignment on the spindle, spindle checkpoint function and cytokinesis. Little is known about the role of Aurora kinase C. As many other regulators of mitosis, Aurora kinases are frequently overexpressed in cancer cells. Therefore, these proteins have become attractive targets for the development of new anti-cancer therapies [3, 4].

Several small-molecule inhibitors of Aurora kinases have been developed and some of them have shown clinical efficacy in phase I and II clinical trials [5, 6]. Among those, one of the most advanced compounds in the clinic is danusertib hydrochloride (PHA-739358; Nerviano Medical Sciences, Nerviano, Italy), which has inhibitory activity against all known Aurora kinases [7–9]. Figure 1 illustrates the molecular structure of danusertib.

We carried out a multi-centric, multi-tumour phase II trial to prospectively assess the anti-tumour activity of danusertib in patients with breast (BC), ovarian (OC), pancreatic (PC), colorectal (CRC), small-cell (SCLC) and non-small-cell lung (NSCLC) cancer, including a NSCLC cohort selecting for squamous cell carcinomas. Secondary end points of this study included an evaluation of the safety profile of danusertib, the monitoring of pharmacokinetics (PK) of the drug in plasma and an exploratory assessment of gene expression profiles in tumour biopsies with potential use for response prediction and patient selection in future trials. The latter results will be reported in a separate publication.

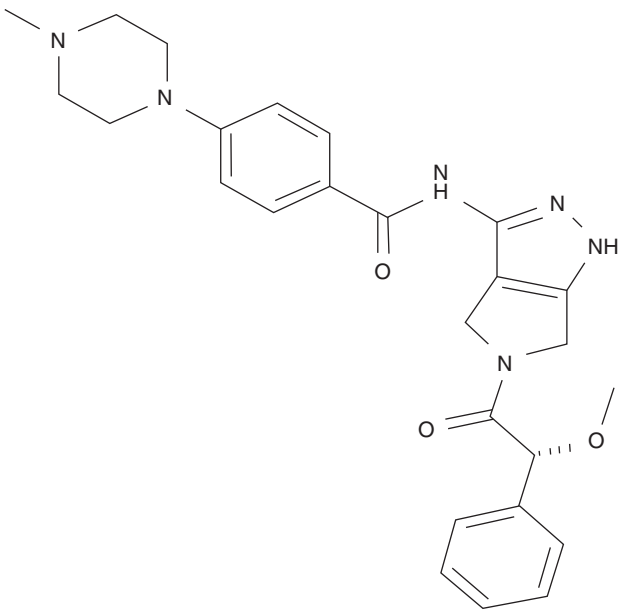

Figure 1. Molecular structure of danusertib.

patients and methods

study design

This prospective phase II, open-label study was conducted at 22 investigational sites in 7 European countries and carried out in accordance with the Declaration of Helsinki, the ICH Harmonised Tripartite Guideline for Good Clinical Practice and national regulatory requirements. All patients provided written informed consent. Patients received danusertib, which was provided by the study sponsor, Nerviano Medical Sciences S.r.l. (Nerviano, Italy).

Adult patients with progressive, locally advanced and/or metastatic BC, OC, CRC, PC, SCLC or NSCLC who had failed second to third lines of palliative treatment as defined per tumour type, were treated with danusertib as a 24-h i.v. infusion every 14 days at a starting dose of 500 mg/m^2, based on results of a phase I trial [10]. The protocol allowed a dose escalation for patients without relevant toxicity in the first treatment cycle and dose reductions in case of adverse events (AEs) during treatment.

For each independent tumour type, a Simon's two-stage design was applied, allowing early termination at completion of the first stage in case of low activity of danusertib in a given tumour type. The primary end point of the study was the progression-free rate (PFR) at 4 months, i.e. the proportion of patients who were still alive and had not progressed during the first 4 months from treatment start. Patients were followed for AEs from informed consent until 28 days after the last dose of study treatment or until a new anti-cancer therapy was started. Safety assessments (vital signs, haematology, blood chemistry and urinalysis) were carried out at baseline, at pre-defined time points during the treatment period and at the end of the experimental treatment. Electrocardiogram (ECG) monitoring and QT interval/corrected QT (QTC) interval assessment were done at baseline, in cycles 1 and 2, and at the end of treatment. A transthoracic echocardiogram or multi-gated acquisition scan were scheduled at the baseline visit and at the end of treatment, to monitor the left ventricular ejection fraction (LVEF).

The RECIST 1.1 efficacy assessment [11] was primarily based on computed tomography (CT) and X-ray investigations; magnetic resonance imaging and positron emission tomography were optional. Imaging was carried out at baseline (within 21 days before the initial infusion treatment) and repeated every 2 calendar months during treatment, with a mandatory scan at the 4 months visit. CT scans of patients with squamous NSCLC were peer-reviewed. Serial tumour marker assessments included the evaluation of CA 125, CEA and CA 19-9, depending on the histological entity, and were done at the same time points as the imaging studies. The safety assessment was based on vital signs, laboratory parameters and cardiac function tests and used CTCAE version 3.0.

Plasma samples were collected before the start of the infusion and just before (5–10 min) the end of infusion on day 1 of treatment cycles 1, 2, 4 and 8 for PK purposes. Danusertib and its N-oxide metabolite PHA-816359 were assessed in plasma using a validated LC-MS/MS method [12, 13]. As exploratory end points protein and gene expression were assessed in pre- and post-treatment tumour biopsies in a subset of selected NSCLC patients. Results of this analysis will be reported in a separate publication.

A Simon's two-stage design was used per tumour type to determine whether danusertib had sufficient activity to warrant further clinical testing [14]. The two-stage procedure allowed the early termination of a given independent disease-specific cohort in case of low activity of the experimental compound. The design was based on testing the null hypothesis, H0: $P \leq p_0$,

Table 1. Assumption of the Simon's two-stage design for this trial

$\alpha = 0.1$ $\beta = 0.1$	Tumour type					
	BC 3rd line	OC 3rd line	PC 2nd line	CRC 3rd line	SCLC 2nd line	NSCLC 2nd line
Median time of PFS for p_0 (months)	1.7	2.8	2.3	2.0	1.7	1.7
PFR at 4 months according to p_0 (%)	20	37	30	25	20	20
PFR at 4 months according to p_1 (%)	40	57	50	44	40	40
Median time of PFS for p_1 (months)	3.0	5.0	4.0	3.4	3.0	3.0
n_1	19	24	28	22	19	19
r_1	3	9	7	5	3	3
n	36	42	39	43	36	36
R	10	19	15	14	10	10
$(r + 1)/n$ (%)	30.5	47.6	41.0	34.9	30.5	30.5

The study design was based on testing a null hypothesis, H0: $P \leq p_0$, that the true progression-free rate (PFR) at 4 months was less than or equal to some uninteresting level p_0, against an alternative one that the true PFR achieved at least some desirable target level p_1. At first stage, Simon's design aimed at stopping the trial if the alternative hypothesis, H1: $P \geq p_1$, could be rejected. In this trial, α and β were taken both equal to 0.1 and the following table presents, for each tumour type, the parameters p_0 and p_1 (tumour type distinctive) used in the set of hypotheses, the required number of assessable patients to be recruited at each stage and the critical number of responses to proceed to second stage and to reject null hypothesis at second and final stage. For each tumour type, the median progression-free rate (PFR) retrieved from literature was used to estimate the PFR at 4 months under the null hypothesis. The computation was done assuming an exponential distribution of the survival function (i.e. constant hazard rate over time).
PFS, progression-free survival; PFR, progression-free rate; p_0, uninteresting level of activity; p_1, interesting level of activity; n_1, first-stage sample size of assessable patients; r_1, upper limit for first-stage rejection of drug; n, maximum sample size of assessable patients; r, upper limit for second-stage rejection of drug; $(r + 1)/n$, lowest percentage of progression-free patients at 4 months by which p_0 is rejected at the end of second stage.

that the true PFR at 4 months was less than or equal to some uninteresting level p_0, against the alternative hypothesis that the true PFR achieved at least some desirable target level p_1. At first stage, the Simon design aimed at stopping the trial if the alternative hypothesis, H1: $P \geq p_1$, could be rejected. In this trial, α and β were defined as 0.1. Table 1 presents for each independent tumour type the p_0 and p_1 assumptions, the required number of assessable patients to be recruited at each stage and the critical number of responses to proceed to the second stage and to reject the null hypothesis. For each tumour type, the median PFR retrieved from literature was used to estimate the PFR at 4 months under the null hypothesis. The computation was done assuming an exponential distribution of the survival function (i.e. constant hazard rate over time). Based on these assumptions, the numbers of patient treated/assessable for each tumour type were the following: 42/38 patients for BC, 34/33 for OC, 33/28 for CRC, 36/30 PC, 18/12 for SCLC and 56/48 for NSCLC. PFR was assessed per disease-specific cohort at the end of each of the two steps of the trial. Point estimates were presented along with two-tail 95% confidence intervals (95% CIs). Summary descriptive statistics including range and median values were estimated according to the Kaplan–Meier method for time-related. Descriptive statistical analyses and individual data listings were produced for the analysis of patient disposition, protocol deviations, baseline characteristics and safety data.

Nerviano Medical Sciences S.r.l. sponsored this trial and provided financial support. All authors contributed to data collection, analysis or interpretation. PS was responsible for drafting, editing and finalizing the manuscript, with input provided by all authors.

results

patient population and tumour characteristics

A total of 223 patients were enrolled in this trial and 219 patients were treated. Table 2 summarizes the characteristics of the enrolled population. Four entered patients, one with CRC, one with PC and two with NSCLC, were never treated. The vast majority of patients (93.6%) had metastatic disease. The most frequent sites of metastasis were lymph nodes and liver and the majority of patients had two disease recurrences/progressions before entering the study. The mean age at study entry was 59.2 years, and most patients (92.2%) were Caucasian. Female patients were slightly overrepresented (59.8%), and there were an equal number of patients with Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1 in this trial (47.9% and 51.1%, respectively).

danusertib treatment

The median absolute and relative dose intensity of danusertib were similar for all tumour types, ranging from 211.4 to 249.1 mg/m^2/week and from 84.6% to 99.6%, respectively. The median number of biweekly treatment cycles delivered ranged from 3 to 4 per patient with a maximum ranging from 5 to 40 courses, depending on the tumour type. Treatment modifications consisted mainly in dosing delays and infusions not completed as per protocol. The most frequent reasons for dose delays were haematological toxicity and logistic reasons. Only seven patients were treated with the allowed increased dose of 580 mg/m^2 after completing the first cycle. The most frequent reason for treatment discontinuation was disease progression (189 patients, 86%); treatment was discontinued due to AEs in only 5.5% of patients.

treatment efficacy

Danusertib did not meet protocol criteria to conclude for clinically relevant activity in any of the tumour types investigated in this trial. In BC, 7 of 38 assessable patients were free from

Table 2. Patient and tumour characteristics and treatment history

Tumour type	BC ($N = 42$)	OC ($N = 34$)	CRC ($N = 33$)	PC ($N = 36$)	SCLC ($N = 18$)	NSCLC ($N = 56$)	All ($N = 219$)
Age (years)							
Mean	53.2	59.0	60.5	59.8	60.7	62.2	59.2
Range	36–70	35–76	38–77	39–80	49–75	39–79	35–80
<65 years	40	23	24	24	13	36	160
≥65 years	2	11	9	12	5	20	59
Sex							
M/F	0/42	0/34	23/10	16/20	12/6	37/19	88/131
Race							
Caucasian/other[a]	39/3	34/0	32/1	35/1	13/5	49/7	202/17
Performance status							
0	23	19	21	13	9	20	105
1	19	15	12	21	9	36	112
2	0	0	0	2	0	0	2

Tumour type	BC ($N = 42$)		OC ($N = 34$)		CRC ($N = 33$)		PC ($N = 36$)		SCLC ($N = 18$)		NSCLC ($N = 56$)		All ($N = 223$)	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Extent of disease														
Metastatic	42	100	32	94.1	33	100	34	94.4	17	94.4	47	83.9	205	93.6
Locally advanced	–	–	2	5.9	–	–	2	5.6	1	5.6	9	16.1	14	6.4
Sites of metastases														
Bone	26	61.9	1	2.9	6	18.2	3	8.3	6	33.3	9	16.1	51	23.3
Liver	23	54.8	7	20.6	27	81.8	25	69.4	8	44.4	15	26.8	105	47.9
Lung	14	33.3	5	14.7	22	66.7	12	33.3	2	11.1	24	42.9	79	36.1
Lymph nodes	15	35.7	26	76.5	17	51.5	13	36.1	13	72.2	34	60.7	118	53.9
Other	10	23.8	23	67.6	8	24.2	11	30.6	6	33.3	19	33.9	76	34.7
No. of recurrences														
1	–	–	1	2.9	1	3	25	69.4	13	72.2	33	58.9	73	33.3
2	8	19.0	25	73.5	18	54.5	10	27.8	5	27.8	19	33.9	85	38.8
>2	34	81.0	8	23.5	14	42.4	1	2.8	–	–	4	7.1	61	27.9
Time from primary diagnosis to treatment start (months)														
Median	76.3	21.2	24	8.1	10.1	11.7	17.5							
Range	9.2–226.7	8.2–163.8	5.6–91.2	3.0–31.8	4.4–41.3	3.2–46.8	3.0–226.7							
Time from current diagnosis of advanced/metastatic to treatment start (months)														
Median	25.5	15.0	17.3	6.0	9.8	7.9	11.3							
Range	3.3–121.1	0.0–34.5	1.1–55.9	0.5–20.6	0.7–41.3	0.2–36.6	0.0–121.1							
No. of prior therapies														
At least 1	42	100	34	100	33	100	36	100	18	100	56	100	219	100
Type of prior therapies														
Systemic	–	–	1	2.9	3	9.1	16	44.4	3	16.7	20	35.7	43	19.6
Systemic + surgery	5	11.9	31	91.2	21	63.6	17	47.2	–	–	6	10.7	80	36.5
Systemic + radiotherapy	2	4.8	–	–	2	6.1	–	–	14	77.8	20	35.7	38	17.4
Systemic + surgery + radiotherapy	35	83.3	2	5.9	7	21.2	3	8.3	1	5.6	10	17.9	58	26.5

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Table 3. Efficacy of danusertib per tumour type—assessable patients

Tumour type	BC ($N = 38$)	OC ($N = 33$)	CRC ($N = 28$)	PC ($N = 30$)	SCLC ($N = 14$)	NSCLC all ($N = 48$)	NSCLC squamous ($N = 31$)
Progression-free rate at 4 months (primary end point)							
No. of patients	7	4	0	3	0	5[a]	5[a]
%	18.4	12.1	0	10.0	0	10.4	16.1
95% CI (%)	7.7–34.3	3.4–28.2	0.0–12.3	2.1–26.5		3.5–22.7	5.5–33.7
Confirmed partial response (RECIST)							
No. of patients	0	1	0	0	0	1[a]	1[a]
Median progression-free survival							
Weeks	8.3	9.3	7.9	8.0	8.1	9.2	9.0
95% CI (weeks)	7.6–9.1	8.7–10.0	7.6–8.9	7.1–9.1	7.1–8.9	18.1–16.6	7.3–16.3
Median overall survival							
Months	12.0	9.8	8.8	4.0	11.4	7.6	7.6
95% CI (months)	9.3–16.1	6.1–14.3	6.0–12.3	2.5–8.6	4.5–n.r.	6.0–10.3	5.6–10.4
50% decrease in serum tumour marker CEA, CA 19-9 or CA 125							
No. of patients	0	3	3	1	0	0	0

[a]Same patient.
n.r., not related.

progression after 4 months of treatment, when compared with the 11 (or more) of 36 required at the end of the second stage to reject the null hypothesis. In OC, 4 of 33 assessable patients were free from progression after 4 months of treatment, when compared with the 10 (or more) of 24 required at the end of the first stage not to reject the alternative hypothesis and to proceed with the second stage. In PC, 3 of 30 assessable patients were free from progression at 4 months precluding the move to the second stage. In CRC, all 28 treated patients assessable for efficacy progressed before the end of the 4th month. In SCLC, none of the assessable patients was free from progression at the month 4 assessment. In non-selected NSCLC patients, the PFR at month 4, observed at the completion of the first stage, did not meet the protocol criteria to proceed with the second stage. In NSCLC selected for squamous cell histology, the PFR at month 4, met the protocol criteria to proceed with the second stage (i.e. at least 3 of 19 assessable patients free from progression at month 4). In the second stage, 5 of 31 assessable patients with squamous histology were free from progression at 4 months.

In the total study population, confirmed RECIST partial responses were limited to one patient with OC (total treatment duration 12 weeks) and one patient with squamous NSCLC (treatment duration 24 weeks). Key efficacy data obtained in this trial are summarized in Table 3. Biochemical responses, here defined as a longitudinal decrease in serum tumour markers >50% when compared with baseline, were seen in three patients with OC, in one patient with PC, in three patients with CRC and in one patient with NSCLC.

safety of danusertib

Overall 219 patients were treated and were assessable for safety. Among these, 216 of patients (98.6%) experienced at least one AE in the first or subsequent cycles. No obvious differences in toxicity were observed among tumour types. The most frequent treatment-related non-haematological AEs (frequency of ≥10%) were fatigue/asthenia (62.1% of patients), nausea (42.0%), diarrhoea (35.2%), anorexia (23.3%), vomiting (21.5%), alopecia (20.5%), constipation (18.3%) and pyrexia (10%).

Neutropenia was the most common haematological toxicity (94.4%) as well as the most frequent grade 3–4 event (82.9%) followed by anaemia, reported in 64.1% of patients. Febrile neutropenia and neutropenic sepsis were documented in 17 (7.8%) and 3 (1.4%) of the 219 treated patients.

Non-haematological laboratory abnormalities were less frequent than haematological events and mainly restricted to mild-to-moderate elevations of liver enzymes. Serum aspartate transaminase and serum alanine transaminase increased in 28.4% and 31.6% of patients, respectively, with grade 3–4 increases in only 3.3% and 3.7% of cases. An increase in alkaline phosphatase was reported in 38.1% of patients (2.8%, grade 3–4), and hyperbilirubinaemia was observed in 11.2% of cases (2.3% grade 3–4).

Drug-related grade 3–4 events occurred in 80 patients (36.5%) and included fatigue/asthenia (28 cases, 12.8%), febrile neutropenia (17 cases, 7.8%), neutropenia (9 cases, 4.1%), anaemia (9 cases, 4.1%), diarrhoea (8 cases, 3.7%), leucopenia (5 cases, 2.3%), neutropenic sepsis (3 cases, 1.4%), chest pain, pyrexia, abdominal pain, urinary tract infection, anorexia, hypertension and transaminases increased (2 cases each, 0.9%), febrile bone marrow aplasia, extravasation, lethargy, mucosal inflammation, abdominal pain upper, dysphagia, vomiting, infection, hypophosphataemia, jugular vein thrombosis, thrombosis, venous thrombosis of the limb, hepatic disorder and function abnormal, headache, pruritus and rash erythematous (1 case each, 0.5%). Hypertension (transient, asymptomatic, reversible, mostly of grade 1–2 in severity) was reported as being drug related in 14 patients (6.6%).

During treatment, no clinically relevant abnormalities in ECG tracing were observed. Ten patients had ECG tracing alterations reported as clinical AE, but only two of these events were considered related to study treatment (namely, grade 1 sinus tachycardia and two episodes of grade 2 atrial flutter in NSCLC

Table 4. Treatment emergent adverse events possibly to definitely related to study treatment occurring in ≥10% of treated patients—all grades, all cycles

Event	BC (*N* = 42)		OC (*N* = 34)		CRC (*N* = 33)		PC (*N* = 36)		SCLC (*N* = 18)		NSCLC (*N* = 56)		All (*N* = 219)	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Any term	39	92.9	34	100.0	27	81.8	32	88.9	15	83.3	49	87.5	196	89.5
Fatigue/asthenia	32	76.2	25	73.5	11	33.3	19	38.9	11	61.1	38	67.9	136	62.1
Nausea	21	50.0	23	67.6	5	15.2	14	38.9	7	38.9	22	39.3	92	42.0
Diarrhoea NOS	18	42.9	15	44.1	12	36.4	13	36.1	3	16.7	16	28.6	77	35.2
Anorexia	8	19.0	7	20.6	6	18.2	11	30.6	3	16.7	16	28.6	51	23.3
Vomiting NOS	9	21.4	12	35.3	5	15.2	8	22.2	4	22.2	9	16.1	47	21.5
Alopecia	13	31.0	8	23.5	3	9.1	7	19.4	1	5.6	13	23.2	45	20.5
Constipation	9	21.4	14	41.2	3	9.1	4	11.1	4	22.2	6	10.7	40	18.3
Anaemia NOS/haemoglobin decreased	6	14.3	10	29.4	3	9.1	9	25.0	2	11.1	7	12.5	37	16.9
Pyrexia	6	14.3	7	20.6	2	6.1	3	8.3	–	–	4	7.1	22	10.0

NOS, not otherwise specified.

Table 5. Haematology toxicity—worst CTC grade emerged or worsened on treatment by patient-treated patients with at least one assessment on treatment

	CTC grade	BC (*N* = 42)		OC (*N* = 34)		CRC (*N* = 33)		PC (*N* = 36)		SCLC (*N* = 18)		NSCLC (*N* = 56)		All (*N* = 219)	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Haemoglobin	*N*	42	100.0	34	100.0	32	100.0	35	100.0	18	100.0	56	100.0	217	100.0
	1–4	28	66.7	27	79.4	15	46.9	19	54.3	13	72.2	37	66.1	139	64.1
	3–4	2	4.8	1	2.9	–	–	3	8.6	–	–	5	8.9	11	5.1
Platelet count	*N*	42	100.0	34	100.0	32	100.0	35	100.0	18	100.0	56	100.0	217	100.0
	1–4	4	9.5	4	11.8	–	–	4	11.4	–	–	4	7.1	16	7.4
	3–4	1	2.4	–	–	–	–	–	–	–	–	1	1.8	2	0.9
WBC	*N*	42	100.0	34	100.0	32	100.0	35	100.0	18	100.0	56	100.0	217	100.0
	1–4	41	97.6	30	88.2	30	93.8	31	88.6	16	88.9	52	92.9	200	92.2
	3–4	25	59.5	23	67.6	21	65.6	13	37.1	14	77.8	31	55.4	127	58.5
Neutrophils	*N*	42	100.0	34	100.0	32	100.0	35	100.0	18	100.0	55	100.0	216	100.0
	1–4	42	100.0	32	94.1	29	90.6	32	91.4	16	88.9	53	96.4	204	94.4
	3–4	36	85.7	30	88.2	29	90.6	27	77.1	16	88.9	41	74.5	179	82.9
Lymphocytes	*N*	42	100.0	34	100.0	30	100.0	35	100.0	18	100.0	55	100.0	214	100.0
	1–4	26	61.9	22	64.7	14	46.7	23	65.7	10	55.6	36	65.5	131	61.2
	3–4	5	11.9	2	5.9	2	6.7	10	28.6	5	27.8	9	16.4	33	15.4

patients). Only two patients in the treated trial population had a QTc interval prolongation, and only two patients had a documented LVEF decrease ≥10% when compared with baseline. None of these observed cardiac function alterations were considered clinically relevant.

In the clinical database, 167 serious adverse events (SAEs) have been recorded in 99 treated patients and 61 (36.5%) of these SAEs, which occurred in 43 patients, were considered as related to the study treatment. Overall, 21 deaths on study (i.e. occurring from patient's consent up to 28 days after last treatment dose) were reported. All events with fatal outcome were assessed by the Investigators as unrelated to the study drug except for two patients who died due to bacterial sepsis and septic shock, both categorized as unlikely being related to the study drug. No grade 5 drug-related events were reported. Almost all patients died due to progression of the underlying cancer or due to events related to their cancer.

The AE profile of danusertib in this multi-tumour phase II trial was similar to safety and tolerability findings in previously reported studies with this agent. Key safety parameters of our trial are summarized in Tables 4, 5 and 6.

pharmacokinetics of danusertib and PHA-816359

Plasma concentrations of danusertib and PHA-816359 were determined in 195 of 219 treated patients. After each cycle of treatment end infusion concentration (C_{endinf}) values of PHA-739358 were comparable among patients with different tumour types while trough concentrations of danusertib were highly variable due to the closeness of the concentrations to the lower

Table 6. Blood chemistry toxicity—worst CTC grade emerged or worsened on treatment by patient-treated patients with at least one assessment on treatment

	CTC grade	BC (*N* = 42)		OC (*N* = 34)		CRC (*N* = 33)		PC (*N* = 36)		SCLC (*N* = 18)		NSCLC (*N* = 56)		All (*N* = 219)	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Albumin	*N*	41	100.0	34	100.0	32	100.0	32	100.0	15	100.0	54	100.0	208	100.0
	1–4	17	41.5	20	58.8	7	21.9	18	56.3	8	53.3	20	37.0	90	43.3
	3–4	–	–	1	2.9	–	–	1	3.1	–	–	1	1.9	3	1.4
Alkaline phosphatase (ALP)	*N*	42	100.0	34	100.0	32	100.0	33	100.0	18	100.0	56	100.0	215	100.0
	1–4	16	38.1	12	35.3	11	34.4	19	57.6	6	33.3	18	32.1	82	38.1
	3–4	1	2.4	2	5.9	1	3.1	2	6.1	–	–	–	–	6	2.8
AST/GOT	*N*	42	100.0	34	100.0	32	100.0	33	100.0	18	100.0	56	100.0	215	100.0
	1–4	14	33.3	10	29.4	11	34.4	12	36.4	4	22.2	10	17.9	61	28.4
	3–4	2	4.8	1	2.9	1	3.1	2	6.1	–	–	1	1.8	7	3.3
ALT/GPT	*N*	42	100.0	34	100.0	32	100.0	33	100.0	18	100.0	56	100.0	215	100.0
	1–4	16	38.1	8	23.5	9	28.1	14	42.4	6	33.3	15	26.8	68	31.6
	3–4	–	–	1	2.9	2	6.3	3	9.1	–	–	2	3.6	8	3.7
Bilirubin total	*N*	42	100.0	34	100.0	32	100.0	33	100.0	18	100.0	56	100.0	215	100.0
	1–4	4	9.5	3	8.8	6	18.8	6	18.2	1	5.6	4	7.1	24	11.2
	3–4	1	2.4	–	–	–	–	3	9.1	–	–	1	1.8	5	2.3
Creatinine	*N*	42	100.0	34	100.0	33	100.0	33	100.0	18	100.0	56	100.0	216	100.0
	1–4	3	7.1	5	14.7	1	3.0	4	12.1	3	16.7	4	7.1	20	9.3
Uric acid	*N*	41	100.0	34	100.0	31	100.0	32	100.0	18	100.0	56	100.0	212	100.0
	1–4	4	9.8	7	20.6	4	12.9	5	15.6	4	22.2	7	12.5	31	14.6
	3–4	–	–	–	–	–	–	1	3.1	–	–	–	–	1	0.5
Calcium	*N*	42	100.0	34	100.0	33	100.0	33	100.0	18	100.0	56	100.0	216	100.0
	1–4	12	28.6	12	35.3	10	30.3	11	33.3	6	33.3	18	32.1	69	31.9
	3–4	1	2.4	1	2.9	–	–	–	–	–	–	1	1.8	3	1.4
Phosphorus, inorganic	*N*	41	100.0	34	100.0	31	100.0	31	100.0	15	100.0	53	100.0	205	100.0
	1–4	8	19.5	8	23.5	7	22.6	6	19.4	4	26.7	20	37.7	53	25.9
	3–4	2	4.9	1	2.9	2	6.5	2	6.5	1	6.7	7	13.2	15	7.3
Potassium	*N*	42	100.0	34	100.0	33	100.0	33	100.0	18	100.0	56	100.0	216	100.0
	1–4	8	19.0	12	35.3	7	21.2	8	24.2	2	11.1	14	25.0	51	23.6
	3–4	–	–	1	2.9	–	–	–	–	–	–	1	1.8	2	0.9
Sodium	*N*	42	100.0	34	100.0	33	100.0	33	100.0	18	100.0	56	100.0	216	100.0
	1–4	9	21.4	12	35.3	10	30.3	14	42.4	8	44.4	28	50.0	81	37.5
	3–4	1	2.4	1	2.9	3	9.1	4	12.1	2	11.1	4	7.1	15	6.9

limit of quantification value. After repeated cycles of treatment, the accumulation ratio of PHA-739358 C_{endinf} was in the range 0.830–0.917 in BC, 1.00–1.42 in OC, 0.824–1.17 in PC, 0.496–1.07 in CRC, 0.796–1.75 in SCLC and 0.871–1.82 in NSCLC patients. Throughout the cycles of treatment, metabolite to parent C_{endinf} ratios were, on average, 0.509, 0.812, 0.460, 0.630, 0.325 and 0.305 in BC, OC, PC, CRC, SCLC and NSCLC patients, respectively. No difference on end infusion concentration of danusertib was apparent among patients with different tumour types. Apart from the higher PHA-739358 C_{endinf} terms of the accumulation ratio range in SCLC (1.75) and NSCLC (1.82) patients, metabolite to parent ratio was similar among patients and approximately equal to 1, indicating, overall, no relevant accumulation of the maximal concentrations of the compound after repeated cycles of treatment. Throughout cycles of treatment, as overall mean, the levels of the metabolite accounted for 50% than those of the parent compound. Cycle 1 concentrations at end infusion of danusertib were similar to that one (mean ± SD: 3.20 ± 1.29 μM) obtained in a previous PK study. Table 7 summarizes the mean ± standard deviation plasma C_{endinf} and trough concentrations of treatment cycles 1, 2, 4 and 8.

discussion

This multi-centric, multi-tumour phase II trial assessed the efficacy and safety of the pan-Aurora kinase inhibitor danusertib in independent cohorts of solid tumour patients after failure of second to third lines of systemic treatment. The 'basket' design of this trial allowed the assessment of activity of the experimental compound simultaneously in several independent disease-specific entities in a very efficient manner.

The safety profile observed was consistent with previous experience with the drug in patients with other solid tumours. No new toxicities were reported and the main target was the haematopoietic system in all tumour subtypes. Neutropenia was the most frequent toxicity (94.4%) as well as the most frequent cause of grade 3–4 toxicities (82.9%). Anaemia and

Table 7. Mean ± standard deviation plasma concentrations at the end of the infusion and trough concentrations of danusertib at cycles 1, 2, 4 and 8

Tumour type	Cycle 1	Cycle 2		Cycle 4		Cycle 8	
	C_{endinf} (μM)	C_0 (μM)	C_{endinf} (μM)	C_0 (μM)	C_{endinf} (μM)	C_0 (μM)	C_{endinf} (μM)
BC	2.65 ± 3.29 (*N* = 35)	0.0331 ± 0.1680 (*N* = 35)	2.43 ± 1.50 (*N* = 36)	0.0014 ± 0.0036 (*N* = 19)	2.20 ± 1.25 (*N* = 22)	0.0294 ± 0.0790 (*N* = 8)	2.36 ± 1.10 (*N* = 7)
OC	3.39 ± 2.78 (*N* = 31)	0.0289 ± 0.0807 (*N* = 28)	3.40 ± 3.37 (*N* = 28)	0.0230 ± 0.0808 (*N* = 21)	3.45 ± 4.35 (*N* = 23)	0.4860 ± 0.8100 (*N* = 5)	4.82 ± 4.70 (*N* = 6)
PC	2.60 ± 2.18 (*N* = 27)	0.0004 ± 0.0009 (*N* = 27)	3.04 ± 3.42 (*N* = 24)	0.0276 ± 0.0987 (*N* = 13)	2.74 ± 2.59 (*N* = 13)	0.0658 ± 0.1140 (*N* = 3)	2.15 ± 0.89 (*N* = 2)
CRC	2.49 ± .930 (*N* = 23)	0.0040 ± 0.0100 (*N* = 20)	2.67 ± 1.96 (*N* = 18)	0.1070 ± 0.3070 (*N* = 10)	1.91 ± 0.99 (*N* = 10)	BLQ (*N* = 1)	(*N* = 1)
SCLC	3.09 ± 3.37 (*N* = 15)	0.0298 ± 0.0746 (*N* = 15)	2.46 ± 1.37 (*N* = 14)	0.0499 ± 0.0943 (*N* = 7)	5.40 ± 7.61 (*N* = 8)	N/A	N/A
NSCLC[a]	3.16 ± 1.95 (*N* = 45)	0.0345 ± 0.1010 (*N* = 42)	5.76 ± 7.24 (*N* = 36)	0.1730 ± 0.5430 (*N* = 28)	4.23 ± 4.14 (*N* = 25)	0.0491 ± 0.0706 (*N* = 12)	2.75 ± 1.41 (*N* = 12)

C_0 = a few minutes before infusion start.
C_{endinf} = 5–10 min before the end of infusion.
[a]All histotypes.
BLQ, below the limit of quantification.

thrombocytopenia, mostly of grade 1–2, were reported only occasionally. Fatigue/asthenia, mainly grade1–2 in severity, was the most frequent drug-related AE in all tumour types, with the highest frequency in BC and OC patients (76.2% and 73.5%, respectively), followed by NSCLC and SCLC (67.9% and 61.1%). Grade 3–4 fatigue/asthenia was uncommon. Mild-to-moderate gastrointestinal AEs were more frequently observed in OC (94.1%) and in BC patients (73.8%), ranging from 51.1% to 63.9% in the other tumour categories tested. The relative absence of effects of the pan-Aurora kinase inhibitor on cardiac function and QTc, and the absence of treatment-related deaths among 219 patients treated in this trial are favourable features of danusertib when compared with other experimental treatments.

PK findings of this trial confirmed the previous experience with danusertib PK in patients with solid tumours. Regarding the maximum concentrations of the compound in plasma after repeated cycles of treatment, the drug was found not to accumulate. Throughout treatment, the levels of the metabolite PHA-739358 accounted for about 50% of the concentrations of the parent compound.

Unfortunately, danusertib monotherapy did not meet protocol criteria to conclude for clinically relevant activity in any of the tumour categories investigated. The number of assessable patients free from progression after 4 months of treatment remained below the threshold defined by protocol. The chosen cut-offs in this trial were based on a thorough analysis of activity of reference treatments in patients with these common cancer types.

There was some indication of clinical activity in patients with NSCLC, OC, PC and BC, with two confirmed objective RECIST responses, some patients with significant decreases in relevant serum tumour markers and a subset of trial participants who remained stable on active treatment of multiple consecutive cycles of i.v. danusertib. In fact, the proportion of patients remaining on active experimental treatment beyond 6, 12 and 24 weeks was 72%, 24% and 9%, respectively.

The unfavourable anti-tumour activity of danusertib observed in this multi-tumour trial is in line with the experience with this and similar cell-cycle modulating agents in oncology. Danusertib showed only marginal activity in solid tumour patients [10, 15] during phase I dose finding, and minimal efficacy in a randomized phase II study in patients with castration-resistant prostate cancer [22]. AT9283, a potent inhibitor of Aurora kinases A and B, achieved only one objective response during phase I dose finding [16]. In a paediatric dose finding trial, the Aurora kinase A inhibitor alisertib achieved an anecdotal partial response in a child with hepatoblastoma. In adult patients with gynaecological malignancies, three patients with platinum-resistant OC achieved objective responses with that compound [17]. Other Aurora kinase inhibitors that were studied in early clinical trials have failed to induce objective responses, such as in the case of the phase I trial with barasetib in solid tumour patients [18].

Not surprisingly, more promising results have been obtained with this haematotoxic class of anti-cancer compounds in patients with haematological malignancies, especially in the case of more promiscuous, multi-targeted Aurora-inhibiting agents and Aurora kinase inhibitors used in combination with cytotoxic agents. The multi-targeted inhibitor MK-0457, that also

inhibits Aurora, was found to be active in BCR-ABL T315I-mutated chronic myelogenous leukaemia, with haematological responses in eight patients, and in three further patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia [19]. When prospectively assessed in a haematological phase I study, MSC1992371A, an oral inhibitor of Aurora and other kinases, induced complete responses in two patients with secondary acute myelogenous leukaemia and in one patient with chronic myeloid leukaemia [20].

In combination with cytarabine, the selective Aurora kinase B inhibitor barasertib achieved an International Working Group Criteria response rate of 45% in haematological tumours [21]. Based on the current clinical experience with various Aurora kinase inhibitors including danusertib, it is unlikely that such promising results in leukaemia can also be obtained in unselected patients with refractory solid tumours after failure of systemic chemotherapy, as tested in the current study.

conclusions

Single-agent danusertib was well tolerated and had favourable PK properties, but showed only marginal anti-tumour activity in heavily pre-treated patients with common advanced solid tumours. Future trials with cell-cycle modulating agents should involve mandatory sequential tissue sampling in all trial participants with accessible lesions and an extensive assessment of biomarkers, to facilitate the identification of predictive factors that could be used for patient selection.

acknowledgements

This study was supported by Nerviano Medical Sciences S.r.l. and conducted by CLIOSS. The authors acknowledge the contribution of all patients and their families to this trial. Furthermore, they are thankful for the active support of this trial by multiple investigators and their trial nursing and data management staff at the involved institutions. PS drafted, edited and finalized the manuscript, with input received from all co-authors. All authors had access to the final study report on the results of this trial.

funding

This work was supported by Nerviano Medical Sciences S.r.l. (no grant number).

disclosure

CD: Employed by Clinical Organization for Strategies & Solutions (CLIOSS) S.r.l., Nerviano Medical Sciences, Nerviano, Italy; MGJ: Employed by Clinical Organization for Strategies & Solutions (CLIOSS) S.r.l., Nerviano Medical Sciences, Nerviano, Italy; AP: Employed by Clinical Organization for Strategies & Solutions (CLIOSS) S.r.l., Nerviano Medical Sciences, Nerviano, Italy. All remaining authors have declared no conflicts of interest with regards to Nerviano Medical Sciences S.r.l. or CLIOSS.

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